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Postnatal ethanol exposure simplifies the dendritic morphology of medium spiny neurons independently of adenylyl cyclase 1 and 8 activity in mice

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Abstract

Background—Fetal exposure to alcohol can have multiple deleterious effects, including learning disorders and behavioral and executive functioning abnormalities, collectively termed fetal alcohol spectrum disorders. Neonatal mice lacking both calcium/calmodulin-stimulated adenylyl cyclases (ACs) 1 and 8 demonstrate increased vulnerability to ethanol-induced neurotoxicity in the striatum compared to wild type (WT) controls. However, the developmental impact on surviving neurons is still unclear.

Methods—WT and AC1/8 knock-out (DKO) mice were administered one dose of ethanol (2.5g/kg) between postnatal days 5-7 (P5-7). At P30, brains were removed and processed for Golgi-Cox staining. Medium spiny neurons (MSNs) from the caudate putamen were analyzed for changes in dendritic complexity; number of branches, branch points and terminals, total and average dendritic length; spine density, and soma size.

Results—Ethanol significantly reduced the dendritic complexity and soma size in surviving MSNs regardless of genotype without affecting spine density. In the absence of ethanol, genetic deletion of AC1/8 reduced the dendritic complexity, number of branch points, spine density and soma size of MSNs compared to WT controls.

Conclusion—These data indicate that neonatal exposure to a single dose of ethanol is sufficient to cause long-term alterations in the dendritic complexity of MSNs and that this outcome is not altered by the functional status of AC1 and AC8. Therefore, although deletion of AC1/8 demonstrates a role for the adenylyl cyclases in normal morphologic development and ethanol-induced neurodegeneration, loss of AC1/8 activity does not exacerbate the effects of ethanol on dendritic morphology or spine density.

Keywords

Fetal Alcohol Syndrome; Dendritic morphology; Caudate Putamen

Introduction

Fetal alcohol spectrum disorders (FASD), affect 2-7 per thousand live births (May *et al.*, 2009). Alcohol exposure during the brain growth spurt period can result in central nervous system damage, which can manifest as behavioral abnormalities, learning disorders, attention deficit disorders, mental retardation, and executive functioning abnormalities (reviewed in (Mattson *et al.*, 2011)). In humans, the basal ganglia, which is comprised largely of the striatum, shows volume reductions in children exposed to alcohol *in utero* (Archibald *et al.*, 2001, Mattson *et al.*, 1996, Roussotte *et al.*, 2012). These deficits in basal ganglia volume, in addition to the effects on the cerebral cortex, hippocampus, thalamus, and cerebellum (Reviewed in (Guerri *et al.*, 2009)), may contribute to impairments in executive and other cognitive functions associated with FASD (Mattson *et al.*, 1996, Riley and McGee, 2005).

The window of rat and mouse brain development from birth until 2 weeks of age is analogous to a similar period in human development that occurs 7-9 months into gestation, a time highly-relevant for the elucidation of fetal alcohol effects (Dobbing and Sands, 1979). Preclinical models of FASD have shown that neonatal rodents acutely exposed to ethanol (EtOH) during this brain growth spurt period demonstrate extensive apoptosis throughout the developing nervous system (Ikonomidou *et al.*, 2000, Olney *et al.*, 2002, Conti *et al.*, 2009b, Maas *et al.*, 2005a, Young and Olney, 2006). Specifically, administration of EtOH during the brain growth spurt period in neonatal rodents (postnatal day 5-7; P5-7) results in significant neuronal death in the striatum as early as 4h after a single EtOH exposure (Conti *et al.*, 2009b, Young and Olney, 2006) while other brain regions, such as the thalamus, cortex, and subiculum demonstrate delayed neurodegenerative profiles after administration of EtOH in 2 doses, 2 h apart (Maas *et al.*, 2005a).

Beyond the established sensitivity to EtOH-induced neurodegeneration, the brain growth spurt period is characterized by tremendous dendritic growth as synapses are formed (Cline, 2001, Dobbing, 1974). Thus, exposure to EtOH during this period may have significant consequences on dendritic morphology, both acutely and in the delayed post-exposure period. Studies of EtOH exposure during the brain growth spurt period in rats have shown changes in both dendritic complexity and spine density in various regions of the brain, although the exact changes vary according to the neuronal subtype. For example, Whitcher and Klintsova demonstrated postnatal binge-like EtOH exposure in rats (5.25 g/kg/day, P4-9), reduced the spine density of layer III neurons in the medial prefrontal cortex at P26-30 (Whitcher and Klintsova, 2008). Also, Hamilton *et al.* found a significant decrease in the number and length of dendrites and an increase in mature spine density of layer II/III pyramidal neurons in the medial prefrontal cortex of P30 rats that were treated with 5.25 g/kg EtOH at P4-9 (Hamilton *et al.*, 2010b). Recent studies on neuronal morphology in the striatum have focused on gestational EtOH exposure (Rice *et al.*, 2012, Lawrence *et al.*, 2012) and only found a significant decrease in dendritic morphology in the nucleus accumbens (NAc) shell (Rice *et al.*, 2012). Despite the striatum's sensitivity to acute postnatal EtOH-induced neurodegeneration (Conti *et al.*, 2009b), to date, little is known about the long-term dendritic response of medium spiny neurons (MSNs) in the caudate

putamen (CP) to a single EtOH exposure during the third trimester equivalent or the mechanisms that mediate these effects.

The actions of EtOH are largely attributed to its antagonism of NMDA receptors and potentiation of GABA_A receptors (reviewed in (Ron, 2004, Ueno *et al.*, 2001)). Antagonism of NMDA receptor-mediated calcium entry may result in impairment of intracellular signaling pathways (reviewed in (Redmond and Ghosh, 2005)), such as those involving the calcium/calmodulin-stimulated adenylyl cyclases (ACs). AC1/8 serve to catalyze cAMP production and are the only two calcium/calmodulin-stimulated AC isoforms expressed in the brain (Wong *et al.*, 1999). Mice lacking AC1/8 demonstrate no measurable calcium-stimulated AC activity in membrane preparations from hippocampus, cerebellum, or whole brain at calcium concentrations up to 100 μ M (Wong *et al.*, 1999). AC1/8 are widely expressed in the developing striatum (Conti *et al.*, 2009b), poising them to be critical modulators of EtOH action in this brain region. Previous work using HEK 293 cells transfected with AC1 failed to show a direct effect of 200mM EtOH on cAMP production (Yoshimura and Tabakoff, 1995) and Maas *et al.* (2005) has also shown AC1/8 enzyme activity to be unaffected by 50mM EtOH using cortical, hippocampal, and cerebellar membranes. However, we and others have previously demonstrated that genetic deletion of AC1 and/or AC8 exacerbates EtOH-induced neurodegeneration in the CP and surrounding subcortical and cortical regions of neonatal mice in the post-treatment period (Conti *et al.*, 2009b, Maas *et al.*, 2005a). More specifically, phosphorylation of the extracellular related kinase, which is involved in the regulation of dendritic development and soma size (Kumar *et al.*, 2005), is impaired acutely in AC1/8 knock-out (DKO) mice following EtOH exposure (Conti *et al.*, 2009b). Roles for AC1/8 in EtOH action are not limited to the neonate. In adult animals, AC1/8 mediate the homeostatic response to EtOH exposure by activating the PKA pathway. (Conti *et al.*, 2009a, Maas *et al.*, 2005b). Presynaptic vesicle-related proteins are phosphorylated after EtOH exposure in WT mice, whereas phosphorylation of these proteins is impaired after EtOH exposure in DKO mice. Further studies using FM1-43 uptake measurements in neurons from the hippocampus of DKO mice revealed fewer active recycling vesicles and a reduction in active terminals which contribute to the impaired presynaptic response in these mice (Conti *et al.*, 2009a). Despite the recognized increase in sensitivity of mice lacking AC1/8 both as neonates and adults in the acute post-treatment period, little is known about the long-term consequences of neonatal EtOH exposure on surviving MSNs in the absence of AC1/8.

Methods

Animals

All mice were backcrossed a minimum of ten generations to WT C57BL/6J mice from The Jackson Laboratory (Bar Harbor, ME). To generate mice for these experiments, homozygous mutants, DKO, and WT mice were bred in house. Mice were maintained on a 12-h light/dark schedule with *ad libitum* access to food and water. All experiments were performed using male mice P5-7, to ensure that all mice weighed 2.5- 3.0g at the time of treatment. Mice of similar weights were used to account for any potential vulnerability due to differences in brain size. A litter matching approach was used to ensure that every

treatment group was comprised of pups from at least 3 litters and control pups were matched from the same litters. Seven WT and 6 DKO pups were used for each treatment for dendritic analysis, and 5 WT and 5 DKO pups were treated with saline, 6 WT and 5 DKO pups were treated with EtOH for spine density measurements. All mouse protocols were in accordance with the National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee at Wayne State University.

EtOH Treatment

Based on previous studies demonstrating the neurodegeneration caused by a single dose of EtOH (Conti *et al.*, 2009b, Young and Olney, 2006), and the significant potential for lethality in DKO pups treated with higher doses of EtOH (unpublished observations), WT and DKO pups were injected subcutaneously (Conti *et al.*, 2009b) with a single dose of EtOH (2.5g/kg) prepared as a 20% solution using 100% EtOH (Decon Laboratories, King of Prussia, PA) in normal saline. Littermate male pups were injected with corresponding volumes of saline as controls. This single dose of EtOH during P5-7 represents maternal exposure to EtOH during the third trimester in humans. All experimental pups were placed on a heating pad at 31°C (Young and Olney, 2006) away from dams until the EtOH pups regained consciousness (~2h), at which time all pups were returned to their dams. Pups were weaned at P21 and euthanized at P30.

Blood Ethanol Concentration

WT and DKO pups were treated with 2.5g/kg EtOH as described above and were placed on a heating pad at 31°C. Pups were euthanized 45 min after EtOH injection and trunk blood was collected in lithium heparin-coated tubes (Sarstedt, Newton, NC) to evaluate blood ethanol concentration (BEC). Ethanol concentration was determined using the Pointe Scientific Alcohol Reagent (Pointe Scientific, Canton, MI) according to manufacturer instructions. All samples were run in duplicate.

Golgi-Cox Staining

At P30, treated pups were euthanized and brains rapidly removed and processed using the rapid Golgi-Cox kit (FD Neurotechnologies, Elliot City, MD) as previously described (Gibb and Kolb, 1998). Briefly, brains were rinsed in PBS and immersed in A/B solution for 17 days under dark conditions. Brains were then transferred to solution C for at least 48h before sectioning on a cryostat at 100µm. Slices were immediately mounted on gelatinized Superfrost slides (Fisher, Pittsburg, PA), dried, and coverslipped as recommended by the kit manufacturer.

Microscopy and Morphological Analysis

Neuron images were obtained using an Olympus BH-2 microscope equipped with Neurolucida software (MBF Bioscience, Williston, VT). MSNs were sampled broadly from the CP between bregma 0.74 and 1.18mm, as previous studies have shown generalized neurodegeneration after a single neonatal exposure to EtOH in this region (Conti *et al.*, 2009b, Young and Olney, 2006). MSNs were selected based on their distinct cellular morphology. Full impregnation of the branches was identified by the characteristic tapering

and hooking of the end of terminal branches. Only neurons that were completely impregnated, not obstructed by other neurons, and completely visible in the thickness of the section were selected and visualized at 20X for morphological analyses and 40X for spine density measurements. Using NeuroLucida software, progressive images (15-30) were taken at increments of 2 μ m through the z-axis. Using NeuronStudio software ((Kutzing et al., 2010); Mount Sinai Medical School, New York, NY), each neuron was traced for Sholl analysis and analyzed for soma size, dendritic length, number of branches, branch points, and end termini. Soma measurements were obtained by first selecting the neuron image with the largest soma profile. Then the major and minor axes were measured and used to calculate the surface area of the soma. Fig. 1 demonstrates representative MSNs from the CP of A. WT saline, B. WT EtOH, C. DKO saline, and D. DKO EtOH mice (Left), their corresponding tracings from NeuronStudio (Center), and the overlay of Sholl rings (Right). Spine density was determined by counting the number of spines in 3 independent 10 μ m-segments of 2nd or 3rd order branches 50 μ m from the cell soma for each neuron. Fifteen neurons per animal were analyzed for neuron and spine analysis.

Statistical Analysis

Data from each neuron was averaged within an animal before statistical analysis (Hamilton *et al.*, 2010b, Rice *et al.*, 2012). Three-way repeated measures ANOVAs were used for statistical analysis of Sholl and branch order data with Genotype (WT and DKO) and EtOH Treatment (Saline and EtOH) as the between-subjects factors and Distance from the soma or Branch Order used as the within-subjects factors. Two-way ANOVA (Genotype X Treatment) was used for all other dendritic and spine measurements, followed by the Tukey post-hoc test. BEC measurements were analyzed using a student's t-test.

Results

Dendritic morphology analysis

Dendritic Complexity/Sholl Analysis—The mixed-design repeated measures ANOVA used to analyze the dendritic complexity of Golgi-Cox stained neurons found main effects for Treatment [$p=0.022$; $F(1, 16)=6.37$] and Genotype [$p=0.047$; $F(1, 16)=4.61$]. Using the Greenhouse-Geisser estimates of sphericity ($\epsilon = 0.46$) to correct the degrees of freedom, because the assumption of sphericity was violated using Mauchly's sphericity test ($\chi^2 = 80.84$, $p<0.001$), we found an interaction between Sholl ring, Genotype, and Treatment [$p=0.037$; $F(3.18, 51.01)=2.97$]. Further analysis revealed a significant interaction of Sholl ring with Treatment for WT mice, indicating that a single exposure to 2.5g/kg EtOH was sufficient to cause lasting effects on MSNs of WT mice. The number of dendritic tree intersections in WT EtOH neurons was significantly reduced 30-70 μ m from the soma compared to WT saline controls (Fig. 2). Deficiency in AC1/8, in the absence of EtOH, likewise decreased the dendritic complexity of MSNs compared to WT saline controls 50-70 μ m from the soma. No significant interaction of Sholl ring and Treatment was found in the DKO mice ($p=0.056$), however 90 μ m from the soma, the DKO EtOH group demonstrated a trend toward a reduction in dendritic tree intersections compared to DKO saline controls. No difference was observed in intersections at distances greater than 150 μ m from the soma between any groups (data not shown).

Dendritic Branch/Length Analysis—Evaluation of additional measures of dendritic complexity were performed to complement the Sholl analysis. Three-way ANOVA analysis of Branch Order revealed a significant interaction between Branch Order and Treatment [$p=0.035$; $F(2.64, 42.36)=3.278$] using Greenhouse-Geisser estimates of sphericity ($\chi^2 = 80.84$, $p<0.001$, $\epsilon=0.53$). Post-hoc analysis revealed a significant effect of postnatal EtOH Treatment on the number of third and fourth order branches (Fig. 3A). Two-way ANOVA analysis also revealed significant effects of EtOH on the number of branch points [$p<0.001$; $F(1,19)=18.96$] (Fig. 3B) and terminal branches [$p<0.001$; $F(1,19)=18.22$] (Fig. 3C), total dendritic length [$p=0.01$; $F(1,19)=9.97$] (Fig. 3D), and average length per dendrite [$p=0.046$; $F(1,19)=2.70$] (Fig. 3E) irrespective of genotype ($p<0.05$). A significant effect of Genotype was observed for number of branch points [$p=0.039$; $F(1, 19)=5.03$]. Together, these results reflect the reduced complexity demonstrated by Sholl analysis in EtOH-treated mice (Fig. 2).

Soma Size—In order to determine if EtOH influenced soma size in addition to dendritic morphology, soma measurements were analyzed from MSNs in the CP of P30 mice after exposure to 2.5g/kg EtOH at P5-7. A significant main effect of Genotype [$p<0.001$; $F(1,19)=22.74$] was observed indicating that calcium-stimulated adenylyl cyclase activity is required for normal soma development. A significant interaction of Treatment x Genotype [$p=0.045$; $F(1,19)=4.72$] was also observed. Post-hoc analysis revealed the interaction to be caused by a significant decrease in soma size of EtOH-treated WT but not DKO mice.

Spine Density—Analysis of the spine density in WT and DKO animals demonstrated a significant main effect of Genotype on both the 2nd [$p=0.048$; $F(1,20)=4.55$] and 3rd [$p=0.003$; $F(1,17)=12.90$] order branches, with DKO animals having a reduced spine density compared to the WT on both branch types, regardless of treatment (Fig. 5). Exposure to 2.5g/kg EtOH at P5-7 had no effect on spine density. The interaction of Genotype and Treatment failed to reach significance for either branch type.

Blood Ethanol Concentration—To determine if differences in susceptibility to changes in dendritic morphology were related to differences in EtOH metabolism, trunk blood was collected 45 min from a separate group of animals treated with 2.5g/kg EtOH at P5-7. BECs from both WT and DKO animals reached ~250mg/dL (Fig. 6) consistent with previous reports in WT animals (Young and Olney, 2006) with no significant difference between genotypes ($p=0.31$).

Discussion

Prenatal alcohol exposure has been demonstrated to reduce brain volume (Archibald *et al.*, 2001), suggesting not only a decrease in neuron number, but also a decrease in cell size, including dendritic branching. In the current study, we demonstrate that a single dose of 2.5g/kg EtOH during the brain growth spurt period decreased dendritic complexity, number of branches, branch points and terminal branches, total dendritic length, and average length per dendrite of MSNs in mice independently of AC1/8 function (Fig. 2-3). Genetic deletion of AC1/8 conferred a decrease in dendritic complexity, number of branch points, and spine density on 2nd and 3rd order branches of MSNs compared to WT mice when examined

collectively for the main effect of Genotype. EtOH also reduced the average soma size of MSNs of WT mice while DKO mice exhibited a reduction in soma size compared to WT mice, with no further effect of EtOH. These effects are not influenced by differences in EtOH metabolism as the BECs from both genotypes did not differ significantly.

In the present study, we demonstrate a decrease in dendritic complexity and spine density in the absence of EtOH in DKO mice, suggesting that the calcium/calmodulin-stimulated ACs play a critical role in modulating normal neuronal development. This basal reduction in dendritic complexity may underlie the altered EtOH sensitivity of DKO mice to early cell death caused by neonatal EtOH exposure (Conti *et al.*, 2009b, Maas *et al.*, 2005a). A possible explanation for the basal decrease in dendritic morphology observed in the DKO mice is impaired signaling pathway activity following calcium influx, potentially through NMDA receptors or L-type calcium channels. These pathways include the protein kinase A-, calcium/calmodulin-dependent protein kinase- and Ras/mitogen-activated protein kinase-mediated pathways, among others. For instance, these pathways can lead to the phosphorylation of CREB, which can play a critical role in modulating dendritic development (Redmond *et al.*, 2002, Redmond and Ghosh, 2005). Mice lacking either AC8 alone or both AC1/8 have demonstrated compromised phosphorylation of CREB in the hippocampus after restraint stress (Schaefer *et al.*, 2000) or fear memory formation (Sindreu *et al.*, 2007), respectively. Since CREB potently regulates the expression of brain-derived neurotrophic factor (BDNF), a modulator of dendritic growth, this may be a mechanism that subserves the observed reduced dendritic complexity and spine density in DKO mice (Gottmann *et al.*, 2009). Morinobu *et al.* have demonstrated a more direct mechanistic link between adenylyl cyclase and BDNF, by using NKH477, a forskolin derivative that stimulates adenylyl cyclase activity to activate BDNF (Morinobu *et al.*, 1999). Similar mechanisms may be involved in the neostriatum, whereby lack of the calcium/calmodulin-stimulated ACs limit dendritic growth. Future studies employing pharmacological manipulations to activate or inactivate PKA and CREB signaling could be used at various points during development to identify windows of maturation during which AC 1/8 activity is crucial for BDNF expression and normal development.

Our data do not demonstrate a significant simplification of dendritic morphology in EtOH-treated DKO mice compared to EtOH-treated WT mice, suggesting that the loss of AC1/8 does not exacerbate the effects of EtOH on dendritic morphology. However, our data do suggest that the ACs may be involved in the effect of EtOH on the soma size of MSNs in the CP. Few studies have examined the correlation between soma size and dendritic complexity and our data suggest distinct mechanisms may be employed in these processes. This result may suggest that DKO mice are partially protected from the effects of EtOH, in that EtOH reduced the soma size in WT but not DKO mice. This could account for the lack of a further reduction in soma size by EtOH found in this study. However, it must be recognized that the partial effects of EtOH on soma size in DKO mice measured here may represent a floor effect, such that because of the altered baseline in the DKO mice, EtOH is unable to further reduce the soma size in these mice.

Our results in mice exposed to EtOH are consistent with other studies which have shown a decrease in dendritic complexity of MSNs from the NAc shell after gestational exposure

(Rice *et al.*, 2012), various subtypes of cortical neurons after gestational and/or postnatal exposure, (Hamilton *et al.*, 2010a, Hamilton *et al.*, 2010b, Lawrence *et al.*, 2012) and a decrease in dendritic length in hippocampal CA1 neurons after exposure throughout gestation and postnatal development (Gonzalez-Burgos *et al.*, 2006). In contrast, additional studies have failed to show a significant change in dendritic complexity of layer III apical dendrites of the mPFC (postnatal exposure; (Whitcher and Klintsova, 2008) or MSNs in the NAc (exposure throughout gestation and postnatal development; (Lawrence *et al.*, 2012) and the dorsolateral or dorsomedial striatum (gestational exposure; (Rice *et al.*, 2012). These differences are postulated to result from different sensitivities of the regions examined (Hamilton *et al.*, 2010b, Lawrence *et al.*, 2012). Likewise, it must be considered that these discrepancies may be accounted for by differences in the FASD model used such as, prenatal or postnatal, or the duration of exposure may contribute to reported variances. Apart from differences in timing and route of EtOH exposure, another explanation for differential results is the sampling regions. Rice *et al.* sampled from small and very distinct regions of the striatum, and found variable changes between regions, whereas we analyzed neurons throughout the striatum, including both the dorsolateral and dorsomedial regions sampled by Rice *et al.* A broad sampling technique was employed to remain congruent with previous work from our lab and others' that demonstrate generalized neurodegeneration in the CP using this treatment paradigm (Conti *et al.*, 2009b, Young and Olney, 2006).

The development of the dendritic tree also includes the formation and maturation of dendritic spines. Spines are dynamic structures that undergo actin dependent changes in size, shape, and number during development and in response to physiological stimuli, including hormonal fluctuations, neuronal activity, and learning (Yuste and Bonhoeffer, 2001). Our results indicate no effect of EtOH on spine density in the striatum are in line with previous studies demonstrating no effect of gestational exposure on spines in the NAc core or shell, and dorsomedial or dorsolateral striatum (Rice *et al.*, 2012) or of gestational and postnatal EtOH on spine density in the NAc (Lawrence *et al.*, 2012). These results differ from those found in adult animals, where chronic alcohol exposure in alcohol preferring rats significantly decreased spine density in the NAc core and intermittent alcohol exposure decreased spine density in the NAc shell (Zhou *et al.*, 2007). These differences indicate varying sensitivity in the striatum not only regionally, but in regard to maturation state as well.

During the brain growth spurt period, afferent innervation is particularly important for dendritic growth (Cline, 2001). Loss of neuronal activity during this period can lead to lasting deficiencies in dendritic morphology. Afferent activity leads to an elevation in intracellular calcium, which leads to changes in dendritic morphology (reviewed in (Redmond and Ghosh, 2005, Wong and Ghosh, 2002)). Previous studies have shown that the cortex is damaged 12-24h after postnatal EtOH exposure, either by a single inhalation exposure (Heaton *et al.*, 2003) or by two subcutaneous injections (Maas *et al.*, 2005a). Because the cortex is one of the main sources of afferent activity for the striatum, the EtOH-induced damage to the cortex could lead to long-term decreases in the dendritic morphology of the striatum. Future studies on the effect of the ACs and neonatal EtOH on long-term cell

survival in the cortex and striatum could be used to determine the role of afferents on dendritic development and spine density.

The present study contributes to our understanding of the molecular mechanisms involved in the dendritic development and spine density of MSNs, both under normal conditions and those following EtOH exposure. Data from WT mice highlight the potency with which a single EtOH exposure impairs long-term dendritic development in the CP, underscoring the risks associated with alcohol ingestion during pregnancy. These data also suggest distinct mechanisms by which the developing brain responds to EtOH in the acute and long-term period, specifically, that AC1/8 are crucial to the acute neuroprotective mechanisms associated with EtOH exposure, but not the long-term morphological response to EtOH treatment. In addition, these data reveal previously unidentified roles for AC1/8 in the normal developmental regulation of MSNs. These results provide a mechanistic foothold useful for future studies of neuronal development and early-life alcohol exposure.

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Figure 1.

Representative photomicrographs of P30 medium spiny neurons from Golgi-Cox stained sections from A. WT saline, B. WT EtOH, C. DKO saline, D. DKO EtOH mice (Left). Dendritic tracings generated using NeuronStudio software (Middle) demonstrate the soma (arrow head), dendrites, branch points (thick arrow) and terminals (thin arrow). Concentric rings representing 10 m interval distances used for Sholl analysis (Right).

Figure 2.

EtOH reduces dendritic complexity of medium spiny neurons. Sholl analysis of P30 MSNs reveals a significant reduction in average number of dendritic tree intersections with Sholl radii 30-70μm from the soma in P30 WT mice exposed to 2.5g/kg EtOH at P5-7 compared to WT saline controls. Deletion of AC1/8, in the absence of EtOH, reduces dendritic complexity 50-70μm from the soma compared to WT controls, with no further effect of EtOH compared to DKO saline controls. Mean $\pm$ SEM, n=5 per group, * WT Saline vs. WT EtOH $p < 0.05$, # WT Saline vs. DKO Saline $p < 0.05$.

Figure 3.

EtOH reduces features of dendritic branching in medium spiny neurons. A. single exposure to 2.5g/kg EtOH at P5-7 significantly reduces the average number of third and fourth order branches of P30 MSNs compared to saline-treated animals regardless of genotype. Significant effects of Genotype and Treatment were found for the number of branch points (B), with genetic deletion of AC1/8 or a single exposure to 2.5g/kg EtOH at P5-7 resulting in a reduction in branch points. A significant effect of EtOH Treatment was also found for terminal branches (C), total dendritic length (D) and average length per dendrite (E) of P30

MSNs in both WT and DKO mice compared to saline controls. Mean $\pm$ SEM, n=5 per group, * Saline vs. EtOH (both genotypes combined) $p < 0.05$, # WT vs. DKO (both treatments combined) $p < 0.05$.

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Figure 4.

EtOH and AC1/8 knockout reduces soma size of medium spiny neurons. A single exposure to 2.5g/kg EtOH at P5-7 or AC1/8 deletion significantly reduces the average soma size of P30 MSNs compared to WT saline controls. Mean $\pm$ SEM, $n=5$ per group, * WT Saline vs. WT EtOH $p<0.05$, # WT Saline vs. DKO Saline $p<0.05$.

Figure 5.

Spine density of MSNs is reduced in DKO mice. DKO mice have significantly decreased spine density compared to WT controls on both second (**Top**) and third (**Bottom**) order branches of MSNs. No effect of EtOH was found in either genotype. Mean $\pm$ SEM, n=5-6 per group, # WT vs. DKO (both treatments combined) $p < 0.05$

Figure 6.

Genetic deletion of AC1/8 has no effect on EtOH metabolism in neonates. BEC levels are equivalent between WT and DKO mice 45 min after EtOH exposure. Blood samples were obtained 45 min following EtOH (2.5g/kg). Mean $\pm$ SEM, n= 6-7 per group.